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Inhibitors in Haemophilia A Patients:

Prevalence and Correlation with Disease Severity

By

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DEDICATION

This work is dedicated to my family:

Father& mother

Husband and kids

Brothers &sisters

For their strong support &care.

ACKNOWLEDGEMENT

Firstly, thanks to God, for giving us the ability and strength to do anything.

I must express my gratitude and deepest thanks to my supervisor for this research Dr. Maria Satti for her supervision, guidance and encouragement throughout this study.

Also thank and gratitude to haemophilia clinic staff: Ahmed Omer and sister Ikhlas and to all haemophilic patients who participate in this study, and to everyone who helped me in this study.

Abbreviations

TF……..Tissue factor

TFPI. ……Tissue factor pathway inhibitors

FV……..Factor five

FVII……Factor seven

FVIII……..Factor eight

FIX…….Factor nine

FX……..Factor ten

FXIII……Factor thirteen

TM…….Thrombomodulin

APC……Activated protein C

TPA……Tissue plasminogen activators

FDPs……..Fibrin degradation products

VWD……Von Willebrand disease

VWF……Von Willebrand factor

PT……Prothrombin time

APTT……..Activated partial thromboplastin time

BT…….Bleeding time

TCT……Thrombin clotting time

CRM+……..Cross reactive material positive

CRM_.....Cross reactive material negative

HIV……..Human immune deficiency virus

HBV…….Hepatitis B virus

HCV…….Hepatitis C virus

DDVP……..1,8des amino-D-arginine/vasopressin desmopressin

EACA……Epsilon amino coproic acid

APCCs…….Activated prothrombin complex concentrates

GP1b…….Glycoprotein 1 b

RIPA……..Ristocetin induced platelet aggregation

PFA100……Platelet function analyzer

PRP……Platelet rich plasma

PPP……Platelet poor plasma

BU…….Bethesda unit

HTC……Haemophilia treatment centre

Abstract

Background: Inhibitors are antibodies that neutralize coagulation factors. They are immunoglobulin's, arising in congenitally deficient individuals as a result of administration of missing factors. The development of inhibitors remains one of the most serious complications of replacement therapy in haemophilia A patients. Inhibitors makes control of bleeding difficult as they need higher doses of factors or bypassing agents, these are coasty and are not always available. This problem is under estimated and needs further work up to detect the true incidence and the risk factors and their effect on management.

Objectives: To determine the prevalence of inhibitors in patients with haemophilia A and correlate the severity of illness with inhibitors level by measuring FVIII level and FVIII inhibitors.

Design: Cross sectional prospective study

Setting: Haemophilia clinic in Khartoum Teaching Hospital in the period from January to May 2010.

Methods: Eighty patients with haemophilia A were included in the study .All patients were males with different disease severities (mild, moderate and severe). Their ages ranged from 3-60 years. Samples were collected from patients for Factor VIII level and inhibitor quantification. Inhibitor quantification was done by Bethesda assay and expressed in Bethesda unit (BU).

Results: Seventeen patients (21.3%) were found to have inhibitors, fifteen patients with low titre inhibitors (18.8%) and two of high titre (2.5%). Those with high titre inhibitors had more frequent visits to haemophilia clinic and received more units of factor VIII during the last month.

No correlation was found between the severity of the disease and the number of visits during the last year. Inhibitors are most common among severely diseased patients but the correlation between the severity of the disease and the inhibitors was not significant for those with mild and moderate disease.

Conclusion: The prevalence of factor VIII inhibitors in this group of patients with haemophilia A was 21.3% (seventeen patients). Fifteen patients of low titre inhibitors (18.8%) and two of high titre (2.5%).This result was significant as the prevalence of inhibitors was 15-25% in haemophilia A as mentioned in the literature . Inhibitors are most common among severely diseased patients. Those with inhibitors had more frequent visits and receive more amount of factor VIII concentrate during the last month when compared with patients with no inhibitors .

المستخلص

المقدمة: هذه الدراسة أجريت لمعرفة نسبة الاجسام المضادة لدى مرضى الهيموفيليا (أ) (سيولة الدم الناتجة من نقص العامل الثامن للتجلط) .تعتبر الاجسام المضادة من المشاكل الخطرة التى يتعرض لها مريض الهموفيليا حيث تتسبب فى عدم استجابة المريض للعلاج ويحتاج المريض لجرعات مضاعفة من العامل الثامن أو علاجات اخرى غير متوفرة فى أغلب الاحيان. هذه المشكلة لم يتم بحثها في السودان وتحتاج لمزيد من الدراسات لتقييمها ومعرفة اثارها على المرضى وعلاجهم.

الاهداف: قياس نسبة العامل الثامن لمرضى الهيموفيليا، قياس نسبة الاجسام المضادة المتكونة لديهم بعد تعاطى العلاج(العامل الثامن) ومقارنة نسبة الاجسام المضادة مع حدة المرض.

نوع الدراسة: دراسة مقطعية وصفية.

مكان الدراسة: مستشفى الخرطوم التعليمى بعيادة الهيموفيليا فى الفترة من يناير الى مايو سنة عشر وألفين.

منهج البحث: تضمنت الدراسة ثمانين مريضا كلهم من الذكور تتراوح أعمارهم من ثلاث الى ستين سنة كما تتراوح حدة المرض لديهم من خفيف , متوسط أ و حاد. أخذت عينات من الدم واجريت فيها الفحوصات اللازمة لمعرفة نسبة العامل الثامن ونسبة الاجسام المضادة.

النتائج: سبعة عشر مريض وجدت لديهم أجسام مضادة بنسبة 21.1%. خمسة عشر منهم كان مستوى الاجسام المضاده لديهم قليل 18.8% بينما اثنان لديهم نسبة عاليه من الاجسام المضادة.2.5%.ترتفع نسبة الاجسام المضادة لدى المرضى ذوى المرض الحاد بينما لم تثبت العلاقة بين تكون الاجسام المضادة وحدة المرض لدى ذوى المرض الخفيف والمتوسط. المرضى الذين لديهم نسبة عالية من الاجسام المضادة يترددون باستمرار لتلقى العلاج ويحتاجون لكميات كبيرة منه مقارنة بالآخرين.

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Introduction and literature review

Normal haemostasis:

Haemostasisis a number of protective processes that have evolved in order to maintain a stable physiology. Haemostasis refer to the process whereby blood coagulation, initiated and terminated in a tightly regulated fashion, together with the removal of the clot (fibrinolysis) as a part of vascular remodeling. As such haemostasis describes the global process by which vessel integrity and patency are maintained over the whole organism for its lifetime. Haemostatic mechanisms are integrated so that thrombin generation is localized, limited and followed by fibrinolysis and tissue remodeling, which are also localized and limited[(1)].

Normal haemostasis involves five major components, platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels. Following blood vessel injury two processes are activated.

- Platelet adhesion to areas of vascular injury with the subsequentformation of a platelet thrombus.
- Surface or tissue contact mediated activation of coagulation with

Sequential enzymic reactions culminating in the formation of a fibrin thrombus[(2)]. These two processes are interactive, although their dominance varies in different sites of the vascular system. Activation of haemostasis is accompanied by activation of the fibrinolytic system which should eventually

achieve partial or complete removal of the thrombus[3]. Blood coagulation occurs when the enzyme thrombin is generated and proteolyses soluble plasma fibrinogen forming insoluble fibrin polymers or clot. Thrombin generation is a complex net work of interactions with positive and negative feedback loops.

Tissue factor (TF) initiates blood coagulation, it is expressed at biological boundaries such as the skin, organ surfaces, vascular adventitia and epithelial mesenchymal surfaces where it function as haemostatic envelope. This ensures that following disruption of vascular integrity blood is immediately exposed to cells expressing TF, leading to the initiation of blood coagulation. Exposure of the circulating zymogene FVII to membrane bound TF lead to activation of FVII to FVIIa and formation of a very high affinity complex of TF with FVIIa result in activation of FIX and FX. In the absence of its activated cofactor FVa, FXa generate small trace amount of thrombin from prothrombin although insufficient to initiate significant fibrin polymerization,thrombin formed in this initiation stage of coagulation is able to back activate FV and FVIII by limited proteolysis.[1]

In the amplification phase of coagulation ,FVIIIa forms a complex with FIXa , the FX- ase complex (FVIIIa – FIXa) and activate sufficient FXa that in complex with FVa form prothrombinase complex (FVa – Fxa) resulting in explosive generation of thrombin that ultimately leads to generation of a fibrin clot.

A key feature of these processes is the assembly of multiprotein complexes on phospholipid surface provided by cell surfaces, for procoagulant complexes such as FX-ase and prothrombinase, this surface is provided by activated platelets

The coagulation factors are either enzymes precursors or cofactors. All the enzymes except FXIII are serine proteases .i.e. their ability to hydrolyse peptide bonds depends upon the amino acid serine at their active site. The scale of amplification achieved in this system is dramatic e.g. 1mol of activated FXI through sequential activation of FIX, X and prothrombin may generate up to2x ([illegible] mol of fibrin.

Feedback inhibition of coagulation occurs by:

- Tissue factor pathway inhibitor (TFPI)
- Anti thrombin (AT) which form inactive complex as with FIX, FX, FXI and thrombin.
- Thrombin also proteolyticaly activate an anti coagulation pathway (protein C pathway), Thrombomodulin (TM) an endothelial cell surface receptor plays a role in activation of this pathway. In complex with its cofactor ,protein S(ps),APC rapidly inactivates the procoagulant cofactors FVa and FVIIIa by specific proteolysis[1].
- Heparin potentiates the action of anti thrombin.
- Heparin cofactor 2 also inhibits thrombin.
- α2 macroglobulin, α 1 anti trypsin, α2 anti plasmin and C1 esterase inhibitor[3].

Fibrionolysis occurs by fibrinolytic system.Components of the fibrinlytic system include plasminogen and plasmin, several endogenous (tissue or plasma

derived)or exogenous(bacterial or venom derived) plasminogen activators and inhibitors. Generation of fibrin and its binding of tissue plasminogen activators (TPA) lead to increase in the affinity of TPA for plasminogen that result in generation of plasmin at the site of fibrin clot .Plasmin can hydrolyse a variety of substrates including factors V and VIII ,but its major physiological targets are fibrin and fibrinogen ,which are split progressively leading to generation of fibrin degradation products (FDPs) .Fibrinolytic system is inhibited by plasminogen activator inhibitor , (PAT_I) and α2 antiplasmin[(1)].

In the classical pathway formulated toexplain in vitro coagulation testing, initiation of the pathway requires contact reaction between FXII, kallikren and high molecular weight kininogen (MWK) leading to activation of FXI. However, Lack of abnormal bleeding in individuals with hereditary deficiencies of these factors suggests that these reactions are not required for physiological coagulation in vivo. FXI does not seem to have a role in the physiological initiation of coagulation; it has a supplementary role in the activation of FIX and may be important at major sites of trauma or for operation[(3)].

Haemophilia A (classic haemophilia)

Definition:

Haemophilia A is an x-linked hereditary disorder that is due to defective or deficient factor VIII molecule. The estimated incidence is only 1in every 10000 live male birth. It's found in all ethnic groups in all parts of the world[4]

Etiology and pathogenesis:

Haemophilia A is a heterogeneous disorder resulting from defects in the factor VIII gene that leads to a reduction in the circulating level of functional FVIII.The reduction in the activity can be due to a decreased amount of FVIII protein, the presence of functionally abnormal protein or both (5). Coagulation FVIII is a single chain protein with 350,000 Mw coded by 186kb gene located in the Long arm of the X chromosome. FVIII is bound in plasma to VWF. It is synthesized in the liver by hepatocytes. For FVIII to be an effective cofactor for FIX it must be activated by thrombin, a reaction that results in the formation of a heterotrimer composed of A1, A2, and A3-C1-C2 domains of FVIII in a complex with calcium [6].Activated FVIII and activated FIX associate on the surface of activated platelets to form a functional FX –activating complex(tenase or x-ase).It is not surprising that haemophilia A and B have similar clinical presentation ,since both FVIII and IX are required to form x-ase complex. In patient with haemophilia, clot formation is delayed because thrombin generation is markedly decreased; the clot that is formed is friable and easily dislodged leading to excessive bleeding.[7]

Genetics:

Haemophilia A is an x-linked recessive disorder that occurs in males and homozygous females; however mild haemophilia has been described in heterozygous females presumably due to extremely unfavorable lionization

(inactivation of normal x chromosome in most of the cells). Approximately 30% of patients of haemophilia have no family history; their disease presumably can be due to new mutations.

Factor VIII gene is very large, about 186kb with about 9kb of exons,it contain 26 exons and 25 intervening sequences or introns. Specific mutations and alterations that results in haemophilia eg: gene rearrangement, missense mutations (in which there is a single base substitution leading to an amino acid change), nonsense mutations (which result in stop codon), abnormal splicing of the gene, deletion of all or portions of the gene or insertion of genetic elements[8].

One of the most common mutations, accounting for40-50% of patients is a unique combined gene inversion and crossing over that disrupt FVIII gene, many of these patients are susceptible to develop FVIII inhibitors.

Large deletions in FVIII gene are always associated with severe haemophilia and some develop inhibitors[9].

Clinical feature:

Hemophilia is characterized by excessive bleeding into various parts of the body. Soft tissue hematomas and hemoarthroses leading to severe crippling hemarthropathy are highly characteristic of the disease. The disease is classified as:

- Mild: if the factor level 5 – 30% of normal (0.05 – 0.30 u/ml) those develop hemorrhage secondary to trauma or surgery they rarely develop spontaneous bleeding.
- Moderate: 1 – 5% of normal (0.01 – 0.05 u/ml) those also develop hemorrhage secondary to trauma or surgery but they have occasional spontaneous hemorrhage.

- Severe: ≤ 1% of normal (≤ 0.01u/ml) those have spontaneous hemorrhage from early infancy and frequent hemarthrosis[10].

  So the severity and frequency of bleeding are inversely correlated with the residual FVIII level. The most affected area is the strain bearing joints e.g. knees, ankles and elbows, if untreated this intra capsular bleeding causes swelling, pain, stiffness and inflammation. It's not clear why bleeding in hemophilia shows predilection for joints (Tissue factor pathway inhibitor in synovial tissue may be part of the explanation) (TFPI). Muscle bleeding most often affect large load bearing groups of muscles of the thigh, calf, posterior abdominal wall and buttocks, but can occur anywhere. Local pressure effect can cause entrapment neuropathy particularly of femoral nerve with illiopsoas bleeding[1].

  - Hematuria is less common and it can cause clot colic.
  - CNS bleeding is un common but can occur after slight head injury and it's a common cause of death.
  - GIT bleeding and oropharyngeal bleeding are uncommon.
  - Bruising is a feature of haemophilia (A) but it's usually self limiting.
  - Pseudo tumors (blood cysts) are blood cysts that occurs is soft tissues or bones. They are rare but dangerous complication of haemophilia.
  - Mucous membrane hemorrhage is common in haemophilia (epistaxis and hemoptysis).
  - Surgery and open trauma invariably lead to dangerous hemorrhage.

So presentation of haemophilia:

- In neonates: cephalohematoma, prolonged bleeding from umbilical cord and post circumcision.
- In childhood: joint bleeding, bleeding from eruption of primary dentitions and post trauma.
- Mild case may present in late life with severe trauma or surgery [1].

Laboratory features:

Patients with severe hemophilia A characteristically have prolonged APTT (activated partial thromboplastin time. the PT, BT and thrombin clotting time (TCT) are normal although minor increase in BT have been reported by some investigators[11].

The APTT is corrected when haemophilic plasma is mixed with an equal volume of normal plasma. If the haemophilic plasma contains an inhibitor (antibodies against FVIII) the APTT on a similar mixture will be prolonged, although incubation of the mixture for 1 – 2 h at 37C^{o} may be required to detect the prolongation.

A definitive diagnosis of hemophilia A is based on a specific assay for FVIII activity. Functional FVIII coagulant activity is measured by one stage clotting assay based on APTT. Chromo metric assay are used for factor VIII activity.

FVIII antigen is measured by immunologic assays which will defect normal and most abnormal FVIII molecules, if the FVIII antigen level is normal but the clotting activity is reduced, the patient has a dysfunctional FVIII molecule, such patients have antigen –positive haemophilia and also referred to as cross reacting material –positive (CRM+).In other patients both FVIII antigen and activity are nearly undetectable – these Patients are CRM negative.

FVIII activity is expressed as percent of normal or as unit per milliliter of plasma. By definition 1 unit of FVIII is equal to the amount in 1 ml of pooled fresh normal human plasma. Also by definition 1 unit of FVIII/ml is 100% of normal[11].

Prenatal diagnosis and carrier detection:

A careful and complete family history is important for carrier detection. All the daughters of haemophilic father will be obligatory carriers of the hemophilic defect. Carrier detection becomes important when daughter of a known carrier or a female offspring of a patient with hemophilia wishes to become pregnant.

If resources for advanced carrier detection are not available one can take a careful history and measure both FVIII and VWF The ratio of VWF to FVIII is higher in carriers[12].

Therapy:

General: general principle includes:

- Avoidance of trauma.
- Avoidance of aspirin, non steroidal anti inflammatory drugs and other agents that interfere with platelets aggregation.
- Avoid (im) injections.

Hemorrhagic episodes can be managed by replacing FVIII;several plasma products can be used [13].

- Fresh frozen plasma: contain FVIII but the disadvantage is that large volume must be infused to achieve and maintain FVIII level.
- Cryo precipitate can be used to attain normal level of FVIII.
- Several commercial lyophilized FVIII concentrate, now available. FVIII concentrate have been sterilized either by heating in solution, super heating to 80 after lyophilization or by exposure to organic solvents (detergent that inactivate enveloped viruses including HIV,HBV and HCV).
- Plasma derived FVIII concentrate prepared by monoclonal antibody techniques and subjected to one of the procedures mentioned above are highly purified.
- FVIII produced by recombinant DNA techniques is now available and both safe and effective.
- Porcine FVIII is also used and it is useful in patient with FVIIII antibodies, because the antibodies maynot cross- react with porcine FVIII.

- DDAVP (1,8 desamino –D- arginine vasopressin, desmopresin), causes atransient rise in FVIII in normal subjects and those with mild to moderate haemophilia but not in patients with severe haemophilia. The peak response to DDAVP usually occurs 30 – 60 min post infusion, but some patients will not respond.
- Antifibrinolytic agents: e.g. episilon- amino coproic acid (EACA) and tranexamic acid have been used to enhance haemeostasis in patients with hemophilia A. They may be given as adjunctive therapy for bleeding from mucous membranes and dental procedures. The usual dose for adult is 1g four times daily.
- Fibrin glue: fibrin tissue adhesives, it contain fibrinogen, thrombin, and FVIII. It's placed on the site of injury and clotted with thrombin solution containing calcium; as a result the fibrin clot is cross-linked and anchored to tissue.
- Liver transplantation: have been successfully transplanted into patients with haemophilia with resulting cure of hemophilic condition, but it's rare because donors of livers are not available.
- Gene replacement therapy may offer ideal prophylactic treatment for hemophilia A. Haemophilia is an excellent model for gene therapy, because the clinical manifestation is the result of deficiency of a single gene product and only small amount of the product (protein) is required to ameliorate symptoms.

Course and prognosis:

The use of replacement therapy has not been without significant complications. The development of antibodies (inhibitors, circulating anticoagulants) against FVIII is one of the serious complications.

Liver disease resulting from viral hepatitis B&C and HIV, Aids is now leading cause of death in older patients with hemophilia[14].

Haemophilia B

Haemophilia B is a blood clotting disorder caused by a mutation of the Factor IXgene, leading to a deficiency of Factor IX. It is the least common form of haemophilia, rarer than haemophilia A. It is sometimes called Christmas disease after Stephen Christmas, the first patient described with this disease.The factor IX gene is located on the X chromosome (Xq27.1-q27.2). It is an X-linked recessive trait, which explains why, as in haemophilia A, only males are usually affected. 1 in 50,000 males are affected.

Factor IX deficiency leads to an increased propensity for haemorrhage. This is in response to mild trauma or even spontaneously, such as in joints (haemarthrosis) or muscles. The symptoms experienced by those affected are similar to those with Haemophilia A.

The level of Factor IX in the blood determines the severity of Haemophilia B. Factor Levels of less than 1-2% is found in people with severe haemophilia and they experience spontaneous bleeds into joints or muscles.
Moderate haemophilia is found in people with factor levels of 2-5%, they may experience spontaneous bleeds into joints and muscles. Or those with mild haemophilia, who have factor levels of between 5-50%, usually only experience Bleeds flowing trauma or surgery.
The major problem in severe Haemophilia B is painful bleeding into joints, mainly the knees, ankles and elbows. These bleeds may occur spontaneously. If left not treated promptly the bleeds may result in permanent arthritis and disability. Most children and adolescents with severe Haemophilia B receive preventative treatment (prophylaxis) with an intravenous infusion of Factor IX, 2 times a week. This is to prevent spontaneous joint and muscle bleeds.

Factor IX has a longer half life than factor VIII and as such factor IX can be transfused less frequently.

With appropriate management and factor replacement children and adults with inherited bleeding disorders can live active and normal lives[15].

Von Willebrand Disease

Background:

Although referred to as a single disease, von Willebrand disease (VWD) is in fact a family of bleeding disorders caused by an abnormality of the von Willebrand factor (VWF). vonWillebrand disease is the most common hereditary bleeding disorder. First described by Erik Adolf von Willebrand in 1926, von Willebrand disease is a congenital bleeding disorder characterized by a lifelong tendency toward easy bruising, frequent epistaxis, and menorrhagia[16].

Pathophysiology

Von Willebrand disease is due to an abnormality, either quantitative or qualitative, of the von Willebrand factor, which is a large multimeric glycoprotein that functions as the carrier protein for factor VIII (FVIII). Von Willebrand factor is also required for normal platelet adhesion. As such, von Willebrand factor functions in both primary (involving platelet adhesion) and secondary (involving FVIII) hemostasis. In primary hemostasis, von Willebrand factor attaches to platelets by its specific receptor to glycoprotein Ib on the platelet surface and acts as an adhesive bridge between the platelets and damaged subendothelium at the site of vascular injury. In secondary hemostasis, von Willebrand factor protects FVIII from degradation and delivers it to the site of injury.

Von Willebrand factor is composed of dimeric subunits that are linked by disulfide bonds to form complex multimers of low, intermediate, and high molecular weights. The small multimers function mainly as carriers for FVIII.High–molecular weight multimers have higher numbers of platelet-

binding sites and greater adhesive properties. Each multimeric subunit has binding sites for the receptor glycoprotein Ib on non-activated platelets and the receptor glycoprotein IIb/IIIa on activated platelets. This facilitates platelet adhesion and platelet aggregation, making high molecular weight multimers most important for normal platelet function[16].

Von Willebrand disease types:

VonWillebrand disease can be classified into 3 main types.

- Type 1 von Willebrand disease, which accounts for 70-80% of cases, is characterized by a partial quantitative decrease of qualitatively normal von Willebrand factor and FVIII. An individual with type 1 von Willebrand disease generally has mild clinical symptoms, and this type is usually inherited as an autosomal dominant trait; however, penetrance may widely vary in a single family. In addition, clinical and laboratory findings may vary in the same patient on different occasions. Typically, a proportional reduction in von Willebrand factor activity, von Willebrand factor antigen, and FVIII is observed in type 1 von Willebrand disease.
- Type 2 disease accounts for 15-20% of von Willebrand disease cases. Type 2 von Willebrand disease is a variant of the disease with primarily qualitative defects of von Willebrand factor. Type 2 von Willebrand disease can be either autosomal dominant or autosomal recessive. Of the 4 described type 2 von Willebrand disease subtypes (ie, 2A, 2B, 2M, 2N), type 2A von Willebrand disease is by far the most common.
    - Type 2A von Willebrand disease is inherited as an autosomal dominant trait and is characterized by normal-to-reduced plasma levels of factor VIIIc (FVIIIc) and von Willebrand factor. Analysis of von Willebrand factor multimers reveals a relative reduction in intermediate and high molecular weight multimer complexes. The multimeric abnormalities

are commonly the result of in vivo proteolytic degradation of the von Willebrand factor. The ristocetin cofactor activity is greatly reduced, and the platelet von Willebrand factor reveals multimeric abnormalities similar to those found in plasma.

- Type 2B von Willebrand disease is also an autosomal dominant trait. This type is characterized by a reduction in the proportion of high molecular weight von Willebrand factor multimers, whereas the proportion of low–molecular weight fragments are increased. Patients with type 2B von Willebrand disease have a hemostatic defect caused by a qualitatively abnormal von Willebrand factor and intermittent thrombocytopenia. The abnormal von Willebrand factor has an increased affinity for platelet glycoprotein Ib.
- The platelet count may fall further during pregnancy, in association with surgical procedures, or after the administration of desmopressin acetate (DDAVP). Although some investigators found DDAVP to be clinically useful in persons with type 2B von Willebrand disease , studies directed at excluding the 2B variant should be completed before DDAVP is used. Measurements of FVIIIc and von Willebrand factor in plasma vary; however, studies involving the use of titered doses of ristocetin reveal that aggregation of normal platelets is enhanced and induced by unusually small amounts of the drug.
- In patients with the rare type 2M von Willebrand disease, laboratory results are similar to those of certain patients with type 2A von Willebrand disease. Type 2M von Willebrand disease is characterized by a decreased platelet-directed function that is not due to a decrease of high–molecular weight multimers. Laboratory findings show decreased von Willebrand factor activity, but von Willebrand factor antigen, FVIII, and multimer analysis are found to be within reference range.

- Type 2N von Willebrand disease is also rare and is characterized by a markedly decreased affinity of von Willebrand factor for FVIII, resulting in FVIII levels reduced to usually around 5% of the reference range. Other von Willebrand factor laboratory parameters (ie, von Willebrand factor antigen [VWF:Ag], ristocetin cofactor activity) are usually normal. The FVIII-binding defect in these patients is inherited in an autosomal recessive manner. Evaluate patients with FVIII deficiency and a bleeding disorder that is not clearly transmitted as an X-linked disorder or those who respond incompletely to hemophilia A therapy for type 2N von Willebrand disease. Unfortunately, the confirmatory test for type 2N von Willebrand disease is not routinely available, likely resulting in an underestimate of the true frequency of this subtype.

- Type 3 is the most severe form of von Willebrand disease. In the homozygous patient, type 3 von Willebranddisease is characterized by marked deficiencies of both von Willebrand factor and FVIIIc in the plasma, the absence of von Willebrand factor from both platelets and endothelial cells, and a lack of response to DDAVP. Type 3 von Willebrand disease is characterized by severe clinical bleeding and is inherited as an autosomal recessive trait. Consanguinity is common in kindreds with this variant. Less severe clinical abnormalities and laboratory abnormalities may be identified in occasional heterozygotes; however, such cases are difficult to identify. Multimeric analysis of the small amount of von Willebrand factor present yields variable results, in some cases revealing only small multimers[16].

Mortality/Morbidity

VonWillebrand disease is estimated to affect about 1% of the population.The morbidity in individuals with von Willebrand disease varies. Many children with von Willebrand disease are asymptomatic. Some of these children have cutaneous and/or mucus membrane bleeding (eg, easy bruising, epistaxis).

Menorrhagia is a common symptom in females with von Willebrand disease. It occurs in more than 50% of women with von Willebrand disease and may be the only clinical manifestation of the disease. The rare type 3 von Will brand disease can manifest with severe bleeding symptoms similar to severe hemophilia (eg, hemarthrosis, intramuscular bleeding).

Race: No influence of ethnicity on the prevalence of von Willebrand disease has been reported.

Sex: VonWillebrand disease affects males and females in equal numbers.

Age: vonWillebrand disease is a congenital bleeding disorder and can be diagnosed at any age.

Clinical:

- Many children with von Willebrand disease (VWD) are asymptomatic and are diagnosed as a result of a positive family history or during routine preoperative screening (eg, prolonged bleeding time). Importantly, remember that a wide variation in clinical manifestations is observed, even for members of the same family.
- The diagnosis of von Willebrand disease can be challenging and depends on an accurate personal and family bleeding history, as well as demonstration of a low von Willebrand factor (VWF) level.[1]
- The history may reveal the following:
    - Increased or easy bruising
    - Recurrent epistaxis
    - Menorrhagia
    - Postoperative bleeding (particularly after tonsillectomy or dental extractions)
    - Family history of a bleeding diathesis
        - Bleeding from wounds

- Gingival bleeding
- Postpartum bleeding

Causes:

Von Willebrand disease is caused by an inherited defect that results in a deficiency or dysfunction of von Willebrand factor. The gene for von Willebrand factor is on the short arm of chromosome 12. It spans approximately 180 kilobases (kb) and is composed of 52 exons. Exons range in size from 40 base pairs (bp) to 1.4 kb. Various point mutations, insertions, and deletions at the von Willebrand factor locus have been described.

In some cases, von Willebrand disease is believed to result from other pathologic processes; however, because of the relatively high prevalence of von Willebranddisease, its concomitant occurrence with other disease states may be coincidental[16].

Tests and diagnosis:

The diagnostic assessments work-up of VWD can be divided into three steps:

1. Identification of patients suspected for VWD, on the basis of data from personal and family clinical history and results of the screening tests of hemostasis.
2. Diagnosis of VWD with identification of its type.
3. Characterization of the subtype.

Screening tests:

These tests are usually applied to patients with suspected bleeding tendency. The **platelet count** isusually normal, but mild thrombocytopenia may occur in

patients with type 2B. The **bleeding time(BT)** is usually prolonged, but may be normal in patients with mild forms of VWD especially when platelet VWF content is normal. The **prothrombintime (PT)** is normal whereas the **Partialthromboplastintime(PTT)** may be prolonged to a variable degree, depending on the plasma FVIII levels.

Identification of the type

VWF antigen (VWF: Ag) is unmeasurable in type 3VWD, whereas it may be low in type 1 and low or normal in type 2 VWD.

The assay for **ristocetin cofactor activity (VWF:RCo)** explores the interaction of VWF with the platelet GpIb/IX/V complex and is still the standard method for measuring VWF activity. It is based on the property of the antibiotic ristocetin to agglutinate formalin-fixed normal platelets in the presence of VWF. In patients with a normal VWF structure (type 1 VWD), values of VWF: RCo are similar to those of VWF:Ag. Factor VIII coagulant activity (FVIII:C) plasma levels are very low (1–5%) in patients with type 3VWD. In patients with type 1 or type 2 VWD,

FVIII:C may be decreased to a variable extent but sometimes is normal.

The multimeric pattern of VWF can be analyzed by agarose gel electrophoresis. Low-resolution agarose gels distinguish VWF multimers, which are conventionally indicated as high, intermediate and low molecular weight. In type 1 VWD all multimers are present, whereas in type 2A and 2B high and intermediate or high molecular weight multimers, respectively, are missing.

Characterization of the subtype

For a correct diagnosis of patients with sVWD and their correct treatment, other assays are necessary to define specific subtypes.

Ristocetin-induced platelet agglutination (RIPA)

is measured by mixing increasing concentrations of ristocetin and patient platelet-rich plasma (PRP)in the aggregometer. Results are expressed as the concentration of ristocetin (mg/mL) able to induce30% of agglutination. Most VWD types and subtypes are characterized by hyporesponsiveness toristocetin, at variance with type 2B, which is characterized by hyper-responsiveness to ristocetin, because of a higher than normal affinity of VWF for platelet GpIb/IX/V complex.

VWF multimeric analysis with high-resolution agarose gels can allow better identification of type1 and 2 VWD subtypes.

Platelet VWF has an important role in primary hemostasis, because it can be released from alphagranules directly at the site of vascular injury. On the basis of its measurement, type 1 VWD may be classified in three subtypes:

- Type 1 “platelet normal”: with a normal content of functionally normal VWF.
- Type 1 “platelet low”: with low concentrations of functionally normal VWF.
- Type 1 “platelet discordant”: containing dysfunctional VWF in platelets.

Factor VIII binding assay measures the affinity of VWF for FVIII. This assay allows type 2N VWD to be distinguished from mild to moderate hemophilia A. In general, a proportionate reduction of both VWF:Ag and VWF:RCo with RCo: Ag ratio □.0.7suggests diagnosis of type 1. The ratio between FVIII and VWF:Ag is always ≥1 and the severity of type 1 VWD phenotype can usually be evaluated

by performing platelet VWF measurements. If the VWF:RCo : Ag ratio is □.0.7 a type 2 VWD might be present. According to the RIPA method, type 2B VWD can be diagnosed in the case of an enhanced RIPA(□.0.8 mg/mL), while types 2A and 2M are characterized by reduced RIPA (□.1.2 mg/mL). Multimeric analysis in plasma is necessary to distinguish between type 2A VWD (lack of the largest and intermediate multimers) and type 2M VWD(all the multimers

present as in normal plasma).Type 2N VWD can be suspected in case of discrepant values between FVIII and VWF:Ag (ratio 0.7) and diagnosis should be confirmed by a specific test of VWF–FVIII binding capacity(VWF–FVIIIB). Additional tests used in VWD diagnosis include the **closure time (CT)** and assays of VWF activity based on binding to collagen (VWF:CB). The evaluation of CT with PFA-100® (Platelet Function Analyzer) allows rapid and simple determination of VWF-dependent platelet function at high shear stress. This system was demonstrated to be sensitive and reproducible for the screening of VWD, even though the CT is normal in type 2N VWD. However, its utility in the clinical setting remains to be demonstrated. Assays for VWF:CB are also available and the ratio of VWF:CB to VWF:Ag levels appears to be useful for distinguishing between type 1 and 2VWD. These relatively new assays have not been properly standardized yet and are not officially recommended by the Scientific Standardization Committee of the International Society of Thrombosis and Hemostasis. A new ELISA test exploiting the interaction of VWF with plate-immobilized GpIb in the presence of ristocetin seems to be very promising in replacing VWF:RCo; however, its validation remains still to be fully ascertained [17].

Treatments and drugs:

Even though von Willebrand disease is a lifelong condition with no cure, the doctor can treat it effectively. Treatment may vary, depending on the type and severity of the disorder, as well as the response to previous therapy and other medications. The most commonly used treatments for von Willebrand disease include:

Desmopressin (DDAVP)

This medication is administered by injection into a vein or, more commonly, through a nasal spray called Stimate. It is a synthetic hormone, similar to the natural hormone vasopressin that controls bleeding by stimulating the body to release more von Willebrand factor already stored in the lining of blood vessels-thereby enhancing factor VIII levels. DDAVP is usually effective in people with type 1 and some subtypes of type 2 disease. Many doctors consider it the first treatment to use in the management of von Willebrand disease. Some women use the nasal spray at the beginning of their menstrual periods to control excessive bleeding or before a minor surgical procedure.

Replacement therapies

These consist of infusions of prepared doses of concentrated blood clotting factors containing von Willebrand factor and factor VIII. They can be useful in all disease types

Contraceptives

These can be useful for controlling heavy bleeding during the menstrual periods. The estrogen hormones present in birth control pills can boost levels of von Willebrand factor and factor VIII activity. Another option is the placement in the uterus of a progesterone-containing contraceptive device.

Antifibrinolytic or clot-stabilizing medications

These medications, such as aminocaproic acid (Amicar) and tranexamic acid (Cyklokapron), can slow down the breakdown of clot putting a stop to bleeding.

Fibrin sealants

These substances, applied like a glue using syringes, are placed directly on a cut to curtail bleeding (16).

Acquired forms of von Willebrand disease can be observed in the following conditions:

- Wilms tumor
- Congenital heart disease
- Systemic lupus erythematosus

- Angiodysplasia
- Seizure disorders treated with valproic acid
- Hypothyroidism(16)

Inhibitors

Inhibitors are antibodies that neutralize coagulation factors. They are immune globulins, usually IgG, arising in congenitally deficient individuals as a result of administration of missing factors. Inhibitors are most common in patients with severe disease although they can occur in mild cases. The most common acquired inhibitors are those directed against factor VIII but antibodies against other Factors can occur e.g.: VWF and FIX. Approximately 25% of patients with severe Haemophilia A develop inhibitors after initial treatment with factor VIII Inhibitors concentrates. Approximately half of these appear transient and disappear with continued therapy with factor VIII. Therefore the diagnosis of an inhibitor, irrespective of titre, requires active monitoring and treatment to determine whether the inhibitor progresses to a high titre inhibitor or it is transient. The incidence of inhibitors in those with severe Haemophilia B is much less common (4%) but requires more careful individual management in aheamophilia treatment centre (HTC).Anaphylaxis can occur and is an additional complication[(18)]

The risk of formation of an inhibitor is influenced by the type of mutation in the factor VIII gene. Large deletions, inversions and crossing over, and nonsense mutations are associated with the highest risk, probably because the recipient's immune system recognizes the normal factor VIII replacement protein as a foreign molecule. The type of mutation also is associated with the severity of haemophiliaA. Thus, the association between the type of mutation and the development of inhibitors may be confounded by variables related to the

severity of illness, such as age at the first infusion of therapy or the cumulative number of days of replacement therapy[19]

The early diagnosis of factor VIII inhibitors is essential. While the presence of an inhibitor can be suspected on clinical ground when the patient does not respond to conventional dose of factor VIII, laboratory diagnosis is required for confirmation.

Inhibitor quantification is done by Bethesda assay and expressed in Bethesda unit (BU).An inhibitor is defined as low titre<5BU and high titre>5BU.Most of the high titre patients are high responding or presents anamnestic response.

A low responding antibody remains at a low or moderate level, whereas in high responders, treatment with factors elicits a sharp anamnestic rise after 5 - 8 days. Low responders can be treated by high dose of factorconcentrate while high responders are refractory to replacement therapy and therefore alternative therapies have been developed .Bypassing agents may be used to treat acute haemorrhage, the most common available bypassing agents are human recombinant FVIIa or plasma derived concentrates that contain activated coagulation factors such as FEIBA and Autoplex. Immune tolerance regimens can be used to eradicate high titre antibodies. The diagnosis of an inhibitor should be conducted using Bethesda criteria. After initial diagnosis of a low titre inhibitor in a patient with Haemophilia A, routine doses of factor VIII may be as much as doubled and the clinical response and inhibitor titre monitored. Subsequent poor clinical response or rising titre levels indicate a non-transient inhibitor. The diagnosis of a high titre inhibitor which does not respond to factor VIII should be treated with an alternative product [18].

There is an incidence of inhibitors in those with mild Haemophilia A following treatment with factor VIII concentrates and the guidelines will also apply to those patients. DDAVP is occasionally used in mild to moderate haemophilia but is often ineffective.

The management of patients with Haemophilia A and inhibitors is complex and must be managed by a Haemophilia Treatment Centre (HTC). Major issues with treatment are firstly, the control of acute haemorrhage and secondly the eradication of the inhibitor (tolerisation)[20].

Control of haemorrhage

Products Available

- Factor VIII, either recombinant or plasma-derived
- Recombinant factor VIIa
- Activated prothrombin complex concentrate (eg FEIBA)
- Prothrombin complex concentrate (egprothrombinex-HT)
- Antifibrinolytics (egtranexamic acid)

Note: Porcine factor VIII is no longer being manufactured.

Factor VIII

In the case of life threatening haemorrhage infusion of factor VIII in large doses can be used to swamp the inhibitor. This therapy is mostly used in patients with low responding inhibitors (inhibitor titre is <5 BU/mL after infusion of factor VIII). In patients who are high responders, this treatment may be effective provided the inhibitor level is less than 5 BU/mL. Factor levels should be observed to assess and monitor response. Anamnesis can occur 5-7 days after therapy and make factor VIII ineffective. Recombinant and plasma-derived factor VIII are available[21].

Recombinant Factor VIIa

This product has been widely used and has proved to be highly effective in the management of spontaneous bleeding episodes which are life threatening. Evidence in the literature suggests that it is effective in79-92% of such episodes. In addition, there is evidence that it is effective in over 90% of cases of surgery.

Recombinant factor VIIa is infused as a bolus. Continuous infusion of recombinant factor VIIa may reduce the quantity and cost of treatment but evidence is conflicting. A recent study suggests continuous infusion of50µg/kg/hr is effective in surgery. Antifibrinolytics are administered concurrently. The standard adult dose of recombinant factor VIIa is 90 µg/kg.
However in children the mean half life is substantially reduced to 1.32 hours and thus higher doses of up to 200-250 µg/kg may be required.. There is widespread evidence that recombinant factor VIIa is effective for treatment of all haemorrhage in patients with high responding factor VIII inhibitors[20].

Activated Prothrombin Complex Concentrates (APCCs)
(E.g.FEIBA VH)

Activated prothrombin complex concentrate, such as FEIBA VH, is effective in the treatment of 90% of bleeding episodes, and has been effective in the management of bleeding during major surgery. An effective dose is 60-100units/kg twice per day. The maximum daily dose of FEIBA is 200units/kg/day.
Antifibrinolytic agents, such as tranexamic acid, should not be administered concurrently with FEIBA. It should be noted that FEIBA contains small amounts of factor VIII and therefore may cause elevation of inhibitor titres in some patients. There is a reported high incidence of thrombosis associated with their use; and it is not possible to measure their activity in a standardized way. Nevertheless, these products remain an option for treatment for complex cases in which alternative methods have proved ineffective.
In patients who are having frequent bleeds, a trial of FEIBA as prophylaxis should be considered. The suggested dose is 75-100 units/kg three times a week.

Prothrombinex-HT

Despite general scepticism about the effectiveness of prothrombinex-HT in the management of joint haemorrhage, some patients report benefit and continue to

be treated with this product. There are concerns about the incidence of thrombosis when using repeated high doses, particularly in the presence of liver disease and when used in combination with antifibrinolytics. There is no evidence of efficacy in serious haemorrhages in surgery.

Antifibrinolytic therapy

The recommended dose of tranexamic acid is 80-100 mg/kg/day, with a standard dose being 1g, 8hr given orally (recommended paediatric dose is 35mg/kg/8hr).

Plasmapheresis / Immunoadsorption

Plasmapheresis can be used to reduce inhibitor titres to allow effective therapy with factor VIII. There is as yet little information on the use of immunosuppression in patients with factor VIII inhibitors but some experimental protocols are being proposed. Immunosuppression has been associated with side effects including delayed wound healing and increased susceptibility to infection. Rituximab (anti CD 20monoclonal antibody) therapy may be considered as an adjunct therapy to reduce inhibitor titres.

Tolerisation:

Immune tolerance induction is defined as eradication of an inhibitor by high dose antigen exposure with or without immune modulation therapy. Tolerisation is an active procedure that may involve development of antibodies against IgG factor VIII antibodies. Better results occurs in those patients with a lower age at the start of ITI; shorter elapsed time of inhibitor presence before ITI; lower maximum pre-treatment inhibitor titres and treatment with higher doses of factor VIII[22].

Justification

The development of inhibitors remains one of the most serious complications of replacement therapy in haemophiliaA patients. Patients with inhibitors need higher doses of factors or bypassing agents which is coasty and usually not available. This problem is under estimated and needs further work up to detect the true incidence and the risk factors and their effect on management.

Objectives

Major:

- To determine the prevalence of inhibitors in patients with haemophilia A.
- To correlate the severity of illness with inhibitors level.

Minor:

- To measure FVIII level in patient with haemophilia A.
- To measure FVIII inhibitors in patients with haemophiliaA.

Material and Methods

Study design:

Cross sectional prospective study.

Study area:

Khartoum teaching hospital in haemophilia clinic in the period (January- May) 2010.

Study population:

Inclusion criteria

- All patients attending haemophilia clinic and previously diagnosed as hemophilia A and who accepted to participate in the study.
- All ages will be included.
- All severities (mild, moderate and severe).

Exclusion criteria

Patients who refuse to participate.

Ethical consideration:

Purpose of the study will be explained to the patients, written consent will be filled with patients. All patients will be informed about the results of their investigations to help the treating doctors in their management.

Sample size

80 patients.

Methodology

Quantitative Measurement of factor VIII Inhibitors:

Factor VIII inhibitors are usually time dependent .Thus if factor VIII is added to plasma containing an inhibitor and the mixture is incubated, factor VIII will be progressively neutralized .If the amount of factor VIII added and the duration of incubation are standardized, the strength of the inhibitor may be measured in units according to how much of the added factor VIII is destroyed[18].

Inhibitors quantification will be done by Bethesda assay and expressed in Bethesda unit (BU).

In the Bethesda assay BU is defined as the amount of inhibitors that will neutralize 50% of 1 unit of FVIII content in normal plasma after 2 hours of incubation at 37° c.

Dilutions of the test plasma are incubated with an equal volume of the normal pooled plasma at 37° c. The normal plasma pool is taken to represent one unit of factor VIII. Dilutions of the control normal plasma containing no inhibitors are treated in the same way, and equal volume of normal plasma mixed with buffer is taken to represent 100% value[18].

At the end the residual factor VIII is assayed and inhibitors strength is calculated from the standard graph of residual factor VIII activity versus inhibitor unit.

Reagents:

1. Platelets poor plasma (ppp) from the patient .Blood was collected in containers with trisodium citrate anticoagulant (9 volumes of blood to

1volume of trisodium citrate) , and then hard centrifugation at 3000g for 15 minute was done to collect ppp leaving the red cells and the buffy coat.

2. Glyoxaline buffer.
3. APTT reagent.
4. Factor VIII deficient plasma.
5. Standard plasma (normal plasma pool).

Method:

Pipette into each of a series of plastic tubes 0.2ml of normal pool plasma .Add 0.2ml of glyoxaline buffer to the first tube (this tube serves as 100% value); add 0.2ml 0f test plasma dilutions in glyoxaline buffer to each of the other tubes ,arrange of dilutions should be set up ranging from undiluted plasma to 1 in 50 dilution.

Cap, mix, and incubate all the tubes for 2 hours at37°c. Then perform factor VIII assay in all incubation mixtures. One stage assay of factor VIII based on APTT is done as follow:

Serial dilutions of test and standard plasma in buffered saline in plastic tubes was made (1 in 5, 10,, 20, 40).Add to each dilution factor VIII deficient plasma and perform APTTs according to the laboratory protocol .The clotting time against factor VIII concentration is plotted on semi log paper and the concentration was read .It is important to obtain straight and parallel line if the result is to be accurate. The reasons for non-parallelism are technical errors, activation of plasma by poor collection, low concentration of factor VIII and presence of an inhibitor.

Calculation of the Results and interpretation:

The dilution of test plasma that give the residual factor VIII percentage nearest to 50% is chosen for calculating the strength of inhibitor .If the residual factor VIII activity is between 80%and 100% the plasma sample does not contain an inhibitor .If the residual activity is less than 60% the plasma unequivocally

contain an inhibitor .Values between 60% and 80% are borderline and additional sample is needed before the diagnosis can be established[18].

Results

Table 1 shows age distribution of the study group. Twenty three (28.8%) were below the age of fifteen, forty three (53.8%) were between (15-30) and fourteen (17.5%) were above thirty.

Table 2 shows distribution of severity among the study group.Twenty three Patients (28.7%) were with mild disease, thirty three (41.3%) of moderate disease and twenty four (30%) of severe disease

Table 3 and figure 1 show the prevalence of inhibitors among the study group. Seventeen patients (21.3%) were found to have inhibitors.

Table 4 and figure 2 show the distribution of level of inhibitors among the study group.Fifteen patients of low titre inhibitors (88.2%) and two of high titre (11.8%).

Table 5 and figure 3 shows the number of visits to haemophilia clinic among the study group. Twenty eight patients (35%) have an average of visits per year of less than 12,thirty two (40%) have an average of (12-24) visits per year and twenty patient (25%) visit the clinic more than 24 visit per year.

Table 6 shows severity versus number of visits per year. Those with mild disease 5 out of 23 (21.73%) have a number of visits of less than 12, 13 out of 23(56.5%) have a number of visits between 12-24 and 5 of 23 (21.73%) have more than 24 visits per year. Those with moderate disease 14 out of 33(42.4%) have number of visits less than 12, 9 out of 33(27.3%) have a number of visits between 12-24 and 10 out of 33 (30.3%) have more than 24 visits. Those with severe disease 9 out of 24(37.5%) have a number of visits less than 12, 10 out of

24 (41.7%) have a number of visits between 12-24 and 5 out of 24(20.8%) have more than 24 visits per year (P value>0.005). This correlation is in significant.

Table 7 shows the number of vials (1 vial= 500 unit) of factor VIII taken in the last month. 34 patients (42.5%) have an average of vials (0-1), 30 patients (37.5%) have an average of (2-5), 14 patient (17.5%) have an average of (6-10) and 2 patients (2.5%) have an average of (10-30).

Table 8 shows the severity versus inhibitors .Patients with no inhibitors 17 out of 62(27.4%) are of mild disease, 29 out of 62 (46.8%) of moderate disease and 16 out of 62 (25.8%) of severe disease. Patients with low titre inhibitors 5 of 15 (33.3%) of mild disease and 3 of 15(20%) of moderate disease and 7 of 15 (46.7%) of sever disease. Patients of high titre inhibitors 1of 2 (50%) are of mild disease and 1 of 2(50%) of sever disease. (P value >0.005)This correlation is insignificant.

Table 9 show the inhibitors versus number of vials taken in the last month. Patients with the least number of vials (0-1) are 34 .28 of them (82.4%) are with no inhibitors, 6 0f them (17.6%) of low titre inhibitors and no one with high titre Those who have a number of visits between (2-5) are 29 patients, 24 of them (79.3%) with no inhibitors, 6 of them (20.7%) are of low titre inhibitors and no one with high titre. Those with number of visits between (6-10) are 14, 10 of them (71.4%) are with no inhibitors, 3 of them (21.4%) with low titre and one patient (7.2%) with high titre .Patients with number of vials between (10-30) are two patients one of them with high titre inhibitors (50%) and one with no inhibitors (50%).(P value < 0.005) this correlation is significant.

Table 1: Age distribution of the study group

Age group (Yrs)	Frequency	Percentage (%)
< 15	23	28.8
15-30 yrs	43	53.8
>30 yrs	14	17.5
Total	**80**	**100.0**

Table 2: Distribution of severity among the study group

Severity	Frequency	Percentage (%)
Mild	23	28.7
Moderate	33	41.3
Severe	24	30
Total	**80**	**100.0**

Table 3: prevalence of inhibitors in the study group

Prevalence of inhibitors	Frequency	Percentage (%)
Positive	17	21.3
Negative	63	78.8
Total	**80**	**100.0**

Table 4: Distribution of level of inhibitor among the study group

Inhibitors	Frequency	Percentage (%)
Low titre	15	88.2
High titre	2	11.8
Total	**17**	**100.0**

Low titre (<5BU)

High titre (> 5 BU)

Table 5: Distribution of number of visits per year.

No. of visits	Frequency	Percentage (%)
< 12	28	35.0
12-24	32	40.0
> 24	20	25.0
Total	**80**	**100.0**

Table 6: Severity versus number of visits per year

	Number of visits per year					
Severity	**< 12**		**12-24**		**> 24**	
	No.	**%**	**No**	**%**	**No**	**%**
Mild	5	21.73	13	56.5	5	21.73
Moderate	14	42.4	9	27.3	10	30.3
Severe	9	37.5	10	41.7	5	20.8
Total	**28**		**32**		**20**	

P value = > 0.005

Table 7: Number of vials taken in the last month.

No. of vials	Frequency	Percentage (%)
0-1	34	42.5
2-5	30	37.5
6-10	14	17.5
10 – 30	2	2.5
Total	**80**	**100.0**

1 vial =500 unit

Table 8: Severity versus inhibitors

Severity	Inhibitors					
	No		Low titre		High titre	
	No.	%	No	%	No	%
Mild	17	27.4	5	33.3	1	50.0
Moderate	29	46.8	3	20.0	0	0.0
Severe	16	25.8	7	46.7	1	50.0
Total	**62**	**100.0**	**15**	**100.0**	**2**	**100.0**

P value > 0.005

Table 9: Inhibitors versus number of vials taken in the last month.

Inhibitors	No. of vials taken in the last month							
	0-1		2-5		6-10		10-30	
	No.	%	No.	%	No	%	No	%
No	28	82.4	24	79.3	10	71.4	1	50
Low titre	6	17.6	6	20.7	3	21.4	0	0.0
High titre	0	0.0	0.0	0.0	1	7.2	1	50
Total	**34**	**100.0**	**29**	**100.0**	**14**	**100.0**	**2**	**100.0**

1 vial=500 unit
P value <0 .005

4.1 Discussion

The development of inhibitor remains one of the most serious complications of replacement therapy in inherited coagulation disorders.

This study was designed to assess the prevalence of inhibitors among patients with haemophilia A presenting to haemophilia clinic in Khartoum teaching hospital in the period from January to May 2010.

Inhibitor quantification was done by Bethesda assay and expressed in Bethesda unit (BU). BU is defined as the amount of inhibitors that will neutralize 50% of 1 unit of FVIII content in normal plasma after 2 hours of incubation at 37^0 C.

An inhibitor is defined as low titre<5BU and high titre>5BU. A low responding antibody remains at a low or moderate level, whereas in high responders ,treatment with factors elicits a sharp anamnestic rise after 5 - 8 days .Low responders can be treated by high dose of factor concentrate while high responders are refractory to replacement therapy and therefore alternative therapies have been developed .Bypassing agents may be used to treat acute hemorrhage ,the most common available bypassing agents are human recombinant FVIIa or plasma derived concentrates that contain activated coagulation factors such as FEIBA and Autoplex. Immune tolerance regimens can be used to eradicate high titre antibodies.

Inhibitor should be looked for every six months in haemophilics on regular replacement therapy which is not done in Sudan due to cost. Inhibitor is only looked for after clinical suspicion of its presence due to suboptimal response to treatment.

Eighty patients with haemophilia A were included in this study. As haemophilia A is an X- linked disease all were males. Their ages ranged between 3-60 years.

Twenty three Patients (28.7%) were with mild disease, thirty three(41.3%) of moderate disease and twenty four(30%) of severe disease.

When they were investigated for inhibitors seventeen patients (21.3%) were found to have an inhibitor. Fifteen patients of low titre inhibitors (18.8%) and two of high titre (2.5%).This result is comparable to the prevalence of inhibitors of (15-25%) in haemophilia A as mentioned in the literature .

Inhibitors were detected in 6 of 23 of patient with mild disease (26%) and 3 of 33 (9%) patients with moderate disease and 8 of 24(33.3%) of severely affected patients.(p>o.o5).This correlation of inhibitors with the severity of the disease is significant for severely affected patients. Regarding those with moderate and mild disease this correlation was insignificant as inhibitors is expected to be higher in those with moderate disease. An explanation is that our patients are treated on demand. There is no home therapy and patient have to come to haemophilia centre each time they have a bleed. The development of inhibitors is affected by the rate of exposure to replacement therapy. Frequency of exposure will be determined not only frequency of bleeding but also the geographical distance to haemophilia centre and affordability of transport. It is also determined by the availability of factor concentrate as it is not always available and many patients refuse to receive plasma replacement.

Thirty two patients (40%) had an average of 24 visits per year with bleeding episodes and received treatment with factor VIII concentrate, fresh frozen plasma or cryoprecipitate, and twenty (25%) have more frequent visits>24 .

Those with high titre inhibitors had more frequent visits to haemophilia clinic and received more units of factor VIII during the last month. The two patients with high titre inhibitors receive an average of 30 vials of (500) IU during the last month which is a very large amount in comparison with those without inhibitors. Again this was significant as patient with inhibitors require more amount of replacement therapy because it will be partially or completely neutralized by the antibodies(p value<0.005).

A previous study done in turkey by Hale Oren Yapark and, Dokuz Eylu, reveals that in 58 haemophilia A patients the prevalence of inhibitors was found to be 27% by Bethesda assay method.

Inhibitors were not detected in any of 14 patients with mild disease, present in9 of 27 (33%) patients with moderate disease and 7 of 17(41) patients with severe disease. During follow up for one year the inhibitors were transient in 10 of 16 (17%) and the prevalence of inhibitors 10% at the end of the study [23]. Follow up the patients with inhibitors is important as many inhibitors in patient with mild disease are transient. This was not done in our study.

Another study was done in Iran by R. Sharifian, M. Hoseini, R. Safai, Gh. Tugeh, A.H. Ehsani, M. Lak and M. Jazeb.

1280 hemophilia A patients (age range 9 months-84 years) were evaluated. All patients received several blood products such as fresh frozen plasma (FFP),cryoprecipitate, factor VIII. 635 of 1280 patients (49.6%), 277 patients (21.6%) and 368 patients(28.8%) had severe, moderate and mild disease, respectively. 184 of 1280 patients (14.4%) developed inhibitor. The prevalence of inhibitor for severe, moderate and mild in hemophilia A patients was 22.8%, 9.4%, 3.5% respectively. 41 patients (22.2%) were high responder and 143 patients (77.8%) were low responder [24].

In comparison to our study the prevalence of inhibitors was higher in patient with moderate disease than mild disease which is not found in our study.

Factors that appear to affect inhibitor development include the severity of hemophilia, age, genetics, and, possibly, the type of replacement therapy administered. Recent studies raise the concern that recombinant factor therapies may be associated with more rapid development and higher levels of inhibitors in previously untreated patients. However, results of different studies are often difficult to compare owing to differences in methodology and populations studied. Further studies standardized in design and methods are clearly needed[25].

A comparative study of measurement of FVIII inhibitors using ELISA and the Bethesda assay done in Korea by KIM SY ,KANG SY, LEE WI ,reveals thatFactor VIII inhibitors are produced during or after coagulation factor VIII (FVIII) therapy in hemophilia A patients. These inhibitors are usually detected by a modified Bethesda assay or an enzyme-linked immunosorbent assay (ELISA). In this study, they used the Bethesda assay to determine the incidence of FVIII inhibitors in 75 fresh plasma samples obtained from 50 hemophilia A patients, and then used ELISA and the Bethesda assay to determine the titres of these inhibitors after the samples had been frozen and thawed. The samples from the screening Bethesda assay were centrifuged and stored at -70 degrees C in accordance with the assay guidelines. Subsequently, these samples were thawed and analyzed using ELISA and the Bethesda assay. The incidence of inhibitors in hemophilia A patients was 20.0%. Among the 35 inhibitor-positive samples identified in the screening Bethesda assay, 16 were positive in ELISA while only 4 were positive in the repeated Bethesda assay. In this study, the ELISA technique showed a higher sensitivity than the Bethesda assay in the detection of FVIII inhibitors in samples that were subjected to freezing and

thawing procedures; this was because the Bethesda assay could not identify the FVIII inhibitors that were degraded after freezing and thawing[26].

4.2Conclusion

From this study we conclude that, prevalence of factor VIII inhibitors in patients with haemophiliaA attending haemophilia clinic in the period from January to May 2010 was 21.3% (seventeen patients). Fifteen patients of low titre inhibitors (18.8%) and two of high titre (2.5%).This result was significant as the prevalence of inhibitors was 15-25% in haemophilia A as mentioned in the literature . Inhibitors are most common among severely diseased patients. Those with inhibitors had more frequent visits and receive more amount of factor VIII concentrate during the last month when compared with patients with no inhibitors.

4.3 Recommendations

- Further in depth studies involving more number of patients is needed as developing of inhibitor is an ongoing process.
- Annual screening for inhibitors for every hemophilic patient.
- Follow up of patient with inhibitors is important as many of them are transient and disappear after time and others increase by replacement therapy
- Measurement of inhibitors should be done regularly every six months for those who receive replacement therapy.
- Risk factors for developing inhibitors need to be evaluated and studied including age of first exposure to replacement therapy and type and purity of factor VIII concentrate.
- Genetic studies are recommended to identify those who are at increased risk for developing inhibitors
- Automation for testing of inhibitors and using ELISA is needed to increase the sensitivity and specificity.

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Serial number ☐

University of Khartoum

Medical Postgraduate Studies Board

Department of pathology

Questionnaire

Name:……

Age ☐ **years**

Sex: **Male** ☐ **Female** ☐

Telephone……

Residence:……

Diagnosis:……

Duration of disease ☐ **years**

Number of hospital visits per month ☐ **per year** ☐

Present complaint:……....

Types of medications: Factor ☐ **NovoVII** ☐ **Others** ☐

Number of factor units taken in the last month: ☐ **units**

Investigation:

Factor level ☐

Inhibitor quantification by Bethesda assay ☐ **BU**

Date:…… **Signature:……**